

Applicants: Iva Greenwald and Diane Levitan
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Filed: Herewith
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Amendments to the Specification:

Please insert the following paragraph at page 1, below the title:

This application is a divisional of U.S. Serial No. 09/043,944, filed March 27, 1998, which is a U.S. §371 National Stage application of PCT International Application PCT/US96/15727, filed September 27, 1996, and claims the benefit of U.S. Provisional Application No. 60/004,387, filed September 27, 1995, the contents of which are hereby incorporated by reference.

Please replace the Sequence Listing beginning at page 69 of the specification with the Sequence Listing submitted with the enclosed specification, following the Abstract of the Disclosure.

Please replace the paragraph beginning at page 14, line 5, with the following amended paragraph:

Applicants genetically mapped *sel-12* to the left of *unc-1* X: from hermaphrodites of genotype *sel-12(ar131) dpy-3(e27)/unc-1(e538)*, 1/36 Sel non-Dpy and 18/19 Dpy non-Sel recombinants segregated *unc-1*. To clone *sel-12*, applicants used the well correlated genetic and physical maps in the *sel-12* region to identify cosmid clones that potentially carried the *sel-12* gene (ref. 27 and A. Coulson et al., personal communication). Applicants assayed pools and single cosmids for the ability to rescue the Egl defect of *sel-12 (ar131)* hermaphrodites, using the plasmid pRF4 [*rol-6 (su1006)*] as a dominant cotransformation marker (28). Ultimately, applicants found that pSpX4, containing a 3.5 kb *SpeI/+Xho I* subclone of C08A12 (Subcloned into KS

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Bluescript, Stratagene) completely rescue *sel-12(ar131)*. When this subclone was microinjected at a concentration of 10 µg/ml into *sel-12(ar131)* animals, 6/6 lines all demonstrated rescue of the Egl phenotype. When applicants attempted to obtain transgenic lines carrying pSpX4 using a concentration of 50 µg/ml, applicants obtained F1 transformants but no stable lines perhaps indicating some toxicity of this plasmid at higher concentrations. Applicants used this genomic subclone to screen a cDNA library (~~kindly provided by Bob Barstead~~) and identified one class of clones of 1.5 kb in size. All subcloning, restriction digests, and library screening were done according to standard techniques (29). Applicants sequenced both strands of the cDNA clone after generating systematic deletions using the Erase-a-base system (Promega®). DNA sequence was performed on double stranded templates using Sequenase™ (US Biochemical). The cDNA contained both a poly (A) tail and a portion of the spliced leader sequence SL1 (ref. 30), suggesting it was a full length clone. Applicants confirmed the 5' end of the cDNA by reverse transcription followed by polymerase chain reaction (RT-PCR) (31). The sequence of this full-length cDNA can be found through GenBank under accession number U35660.

Please replace the paragraph beginning at page 14, line 39, with the following amended paragraph:

To identify the lesions associated with *sel-12* alleles applicants used PCR to amplify the *sel-12* genomic fragment from DNA isolated from the *sel-12* mutant strains using the primers DL103 (5'TGTCTGAGTTACTAGTTTCC 3') (SEQ. ID. NO:7) and DLG3 (5'GGAATCTGAAGCACCTGTAAGCAT 3') (SEQ. ID. NO:8). An aliquot of this double-stranded amplification product was

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used as the template in a subsequent round of PCR using only the primer DL103, to generate a single-stranded template. Exon specific primers were used to determine the entire coding sequence for all three alleles. For each allele, only one alteration in sequence was identified.

Please replace the paragraph beginning on page 52, line 31 with the following amended paragraph:

PS1: Full-length human PS1 cDNA and cDNA encoding the PS1 A246E substitution were generated by RT-PCR of cytoplasmic RNA isolated from skin fibroblasts of a patient harboring the A246E mutation (NIA Cell Repository #AG06848B) using a sense primer, hAD3-ATG-Kpn (GGGGTACCATGACAGAGTTACCTGCAC, SEQ ID NO:10), and antisense primer, hAD3-R-3'UTR (CCGGGATCCATGGGATTCTAACCGC, SEQ ID NO:11). PCR products were digested with Asp718 and BamHI and ~1.4 kB hPS1 cDNAs were gel purified and ligated to Bluescript KS+ vector (Stratagene, La Jolla, CA.) previously digested with Asp718 and BamHI, to generate phPS1 and phPS1A246E. The cDNAs were sequenced in their entirety using a Sequenase™ kit (U.S. Biochemical Corp., Cleveland, OH).

Please replace the paragraph beginning on page 53, line 11 with the following amended paragraph:

For M146L, primer pairs were hAD3-M146LF (GTCATTGTTGTCCTGACTATCCTCCTG, SEQ ID NO:12) /hAD3-R284 (GAGGAGTAAATGAGAGCTGG, SEQ ID NO:13) and hAD3-M146LR (CAGGAGGATAGTCAGGACAACAATGAC, SEQ ID NO:14) /hAD3-237F (CAGGTGGTGGAGCAAGATG, SEQ ID NO:15). PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting product was digested with KsI and PflMI and an

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~300 bp gel purified fragment was ligated to KasI/PflMI-digested phPS1 to generate phPS1MI46L. For H163R, primer pairs were hAD3-H163RF (CTAGGTCATCCGTGCCTGGC, SEQ ID NO:16) /hAD3-R284 and hAD3-H163RR (GCCAGGCACGGATGACCTAG, SEQ ID NO:17) /hAD3-237F. PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-237F and hAD3-R284. The resulting products were digested with KasI and PflMI and a gel-purified ~300 bp fragment was ligated to KasI/PflMI-digested phPS1 to generate phPS1H163R.

Please replace the paragraph beginning on page 53, line 28 with the following amended paragraph:

For L286V, primer pairs were hAD3-L286VF (CGCTTTTCCAGCTGTCATTTACTCC, SEQ ID NO:18) / hAD3-RL-GST (CCGGAATTCTCAGGTTGTGTTCCAGTC, SEQ ID NO:19) and hAD3-L286VR (GGAGTAAATGACAGCTGGAAAAAGCG, SEQ ID NO:20) / hAD3 -F146 (GGATCCATTGTTGTCATGACTATC, SEQ ID NO:21). PCR products from each reaction were gel purified, combined and subject to a second round of PCR with primers hAD3-F146 and hAD3-RL-GST. The resulting products were digested with PflMI and BbsI and a gel purified ~480 bp fragment was ligated to PflMI/BbsI-digested phPS1 to generate phPS1L286V.

Please replace the paragraph beginning on page 53, line 39 with the following amended paragraph:

For C410Y, primer pairs were hAD3-C410YF (CAACCATAGCCTATTTTCGTAGCC, SEQ ID NO:22) /LRT7 (GCCAGTGAATTGTAATAGGACTCACTATAGGGC, SEQ ID NO:23) and hAD3-C410YR (GGCTACGAAATAGGCTATGGTTG, SEQ ID NO:24) /hAD3-243S (CCGGAATTCTGAATGGACTGCGTG, SEQ ID NO:25). PCR products from each reaction were gel purified, combined and subject to a

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second round of PCR with primers hAD3-243S and LRT7. The resulting products were digested with BbsI and BamHI and an ~300 bp fragment was gel purified and ligated to BbsI/BamHI-digested phPS1 to generate phPS1C410Y.

Please replace the paragraph beginning on page 54, line 13 with the following amended paragraph:

PS2: Full-length cDNA encoding human PS2 was generated by RT-PCR of total human brain RNA using a sense primer, huAD4-ATGF (CCGGTACCAAGTGTTCGTGGTGCTTCC, SEQ ID NO:26) and antisense primer, hAD4-stopR (CCGTCTAGACCTCAGATGTAGAGCTGATG, SEQ ID NO:27). PCR products were digested with Asp718 and XbaI and ~1.4 kB hPS2 cDNA were gel isolated and ligated to a vector fragment from expression plasmid pCB6⁺ (17) previously digested with Asp718 and XbaI to generate phPS2. The insert was sequenced in its entirety using a SequenaseTM kit (U.S. Biochemical Corp., Cleveland, OH).